NASA FINAL REPORT 1993

Title of Project : "Mutant strains of Anabaean variabilis which excrete ammonia as source of nitrogen for plant growth in CELSS"

Principal Investigator:Muthukumaran Gunasekaran

Address : Department of Biology

Fisk University, Nashville, TN 37208

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Objectives and significance

One of the main factors limiting plant productivity is ther availability of nitrogen. Since industrial fertilizer has a high energy dependence and is often the cause of environmental pollution, researchers are looking for new and cost-effective ways by which dinitrogen can be used to support the growth of crop plants in N-depleted environments. The work carried out by Shanmugam and coworkers clearly showed that genetically modified cyanobacteria can provide at least part of the nitrogen required by the plants using dinitrogen. Since nitrogen fixation genes cannot be directly incorporated into the genome of higher plants, the technologfy described in this proposal offers a viable alternative to linking dinitrogen fixation to the growth of cereal crop plants. During the course of this project, we have applied a co-culture system of wheat (Yecors rojo) with an ammonia-excreting mutant of the cyanobacterium Anabaena variabilis, which can provide N-fertilizer for ther growth of wheat plants in N-free medium. The main objective of this proposed research was to explore the physiological conditions for a successful hydroponic co-culture system and to define some of the exchange processes taking place betweeen wheat roots and cyanobacterial mutant. We further generated similar mutants using nitric acid mutagenesis, and characterized them with respect to ammonia production and some properties of glutamine synthetase, the key enzyme for nitrogen assimilation.

ACCOMPLISHMENTS

Research results during the first year were obtained in trial experiments carried out in the green house using peat pots, growth in 250 ml fleakers using sand as carrier, a 2-cup system with the holding cup containing the cyanobacteria and a hydroponic system where the cyanobacteria were added to the rhizosphere of wheat. The latter system proved to be superior to all other systems in terms of grain yield, nitrogenase assays and the convenience of changing the medium for experimental purposes. Growth conditions were optimized with respect to plant density and light regime in the co-culture. Growth and nitrogenase activity of the free-living strains SA-0 and SA-1 were compared to the co-culture using the same parameters. Biomass and grain yield were determined for all four different growth conditions (see Spiller and Gunasekaran 1990). Wild type strain SA-0 did not support growth of wheat. The size of the inoculum was varied from 2 to 20 mg dry wt SA-1 applied to co-cultures. Grain yield and biomass increased considerable with aigh inoculum of cyanobacterial mutant (Thesis, Ransom 1990).

The experiments of the second year were geared toward in depth study of the interactionof ammonia-excreting mutant SA-1 with the wheat plant with respect to photosynthetic oxygen evolution and resoiration, the effect of low levels of ammoni on the ammonia-excreting properties of the mutant, the quantitiy and timing of cyanobacterium applied and the physiological and enzymatic properties of a selected group of mutants supporting growth o wheat. The effect of light intensity on oxygen evolved was measured 'in situ' and the effect of light intensity on both nitrogenase activity and net oxygen evolution was measured simultaneously in a specially constructed reaction chamber (Spiller and Gunasekaran 1991). In an unstirred co-culture system of wheat with cyanobacterium, oxygen supply by the mutant strain SA-1 to the roots is a key factor in plant productivity.

The type of interaction between plant and microbe was studied by physically separating the mutant from the roots by keeping the cyanobaccterium in a dialysis bag. The experiemnt had four treatments : control plants without inorganic N or cyanobacteria; nitrate added at 2 mM; mutant SA-1 in a dialysis bag; mutant SA-1 in direct association. Growth was monitored as leaf area for 3 weeks, and final cell mass and grain yield determined after an entire growth cycle of 8 weeks. The growth of the wheat plant separated by dialysis membrane was nearly as low as the control without any addition (see accompanying manuscript submitted Spiller et al., 1993). The ammonia-excreting properties of a free-living batch culture were monitored during an entire growth cycle as a function of ammonia (0.5 to 4 mM) added. Whereas nitrogenase activity of wild typwe strain SA-0 was inhibited by 100% at 1 mM ammonia, this activity was at a maximum in the mutant SA-1 under the same conditions. Growth conditions were optimized in a co-culture when several smaller inocula were applied over a period of 3 weeks (see manuscript Spiller et al. 1993). Some physiological properties of other ammonia-excreting mutants were studied with respect to oxygen evolved, root respiration, nitrogenase activity. Biomass and grain yield of the co-culture with the most productive mutants were determined (Thesis, Venson 1991).

During the third year our efforts were directed toward studying the nutrient requirement of both cyanobacterium and wheat in the co-culture, to study the plant/cyanobacterial interaction, study the flow of fixed carbon to the rhizosphere of wheat in the light (for methodology see Spiller and Stallings 1993), and to determine nitrogenase activity in root segments and compare it with the activity of detached filaments, study the possible role of sucrose invertase in roots with and without cyanobacterium. The results of these studies are reported in the accompanying manuscript submitted (Spiller et al. 1993).

Students trained in this program

A. Graduate students

1. Ransom, Maurice

Thesis title: "Growth of a co-culture of wheat with ammonia-excreting mutant of Anabaena variabilis ", graduated May 1990

2.Venson, Pamela

Thesis title: "Physiological characteristics of mutants of Anabaena variabilis with ammonia-excretung properties in a culture supporting the growth of hysroponic wheat." Graduated May, 1992.

3. Taylor, Jacqueline

Thesis title: "Physiological characterization of ammonia-excreting mutants of Anabaena variabilis ". Graduated May, 1992.

B. Undergraduate students, worked in this program, and their special topics

1. Nesbitt, Emma
Project title: "pH changes in hydroponic wheat/Anabaena co-cultures", 1990.
2. Venson, Pamela
Project title : "Oxygen evolution and nitrogenase activity of ammonia-excreting mutants of Anabaena variabilis " ,1990.
3. Stallings, William
Project title : "Growth and nitrogenase activity in dialysis diffusion cultures vs. direct association cultures of cyanobacterium mutant SA-1 with wheat roots", 1990/1991.
4. Woods, Trent
Project title: "The effect of amonia concentration on growth and nitrogenase activity of ammonia-excreting mutant SA-1 of the cyanobacterium Anabaena variabilis ", 1990/1991.
5. Kimberly Looney
Project title: "Invertase activity of wheat roots co-cultured with cyanobacterial mutant SA-1 of Anabaena variabilis, 1993.

Publications and Presentations

1. Spiller H. and Gunasekaran M. (1990). Ammonia-excreting mutant of Anabaena variabilis supports growth of wheat. Appl. Microbiol. Biotechnol. 33:477-480.

2. Spiller H. and Gunasekaran M. (1991). Simultaneous oxygen production and nitrogenase activity of an ammonia-excreting mutant of the cyanobacterium Anabaena variabilis in a co-culture with wheat. Appl. Microbiol. Biotechnol. 35:798-804.

3. Spiller H. and Stallings Jr. W. (1993). An efficient method for the recovery of 14C fromplant parts in labeling experiments of whole wheat. In press 1993.

4. Spiller H., Stallings Jr. W., Woods, T., and Gunasekaran M. Requirement for direct association of ammonia-excreting cyanobacterium and wheat roots is necessary for maximal growth and yield of wheat. Submitted to Appl. Microbiol. Biotechnol. 1993.

5. Ransom, Maurice, M. Gunasekaran, and H. Spiller. "Ammonia-excreting mutant of A. variabilis supports growth of wheat in model for space bioreactor" Am. Soc. for Plant Physiol. Indianapolis IN, 1990.

6. Stallings Jr. W., Woods T., Spiller H. and Gunasekaran M. Direct association of cyanobacterium and wheat roots is necessary for wheat growth in N-deficient, hydroponic system. ASM meeting abstract Dallas, TX 1991.